cells or target particles. As initially applied to devices, i.e., before any recirculation of product, wash fluid should devoid of target cells or particles and without any of the cells or particles of less than the predetermined size.

[0013] The microfluidic device should preferably be configured for deterministic lateral displacement (DLD), a process that involves flowing a sample through a specifically designed array of microposts that are tilted at a small angle from the direction of fluid flow (see, Davis, et al., *Proc. Natl. Acad. Sci. USA* 103:14779-14784 (2006); Inglis, et al., *Lab Chip* 6:655-658 (2006); Chen, et al., *Biomicrofluidics*. 9(5): 054105 (2015)), incorporated by reference herein in their entirety. Thus, the microfluidic device will have an array of obstacles arranged in rows, with each subsequent row of obstacles shifted laterally with respect to a previous row. The obstacles should be positioned so as to deflect target cells or particles to a first outlet (where they may be recovered as a target cell or particle product), and to direct cells or particles of less than the predetermined size to a second outlet (where they may be collected or discarded as waste).

[0014] DLD is performed by flowing the sample and wash fluid through the microfluidic device. During this process, at least a portion of the target cell or target particle product is recirculated from the first outlet to replace all, or at least a portion, of the wash fluid being applied to an inlet of the device. During, or at the end of, the procedure, a final product comprising target cells or particles is obtained from the first outlet.

[0015] To facilitate recirculation, the first outlet on the microfluidic device may comprise, or be connected to, a valve that can be used to divert the target cell or target particle product to a conduit that recycles the product to an inlet on the microfluidic device. Similarly, the microfluidic device may have an inlet that comprises, or is connected to, a valve that can be used to switch the feed entering the device through the inlet from a conduit feeding wash fluid to a conduit feeding target cell or target particle product.

[0016] In some embodiments target cells or target particles being recirculated to a microfluidic plate are reacted with, or bound to, a carrier, antibody, fluorescent tag, activator or compound prior to, during or after being reapplied to the microfluidic device. In a preferred embodiment, target cells, e.g., leukocytes or, more specifically, T cells, are the target cells and are bound with specificity by carriers, antibodies or activators. As used in this context, the word "specificity" means that at least 100 (and preferably at least 1000) target cells will be bound by carrier relative to each non-target cell bound in a sample. Such binding may be used to promote cell division (in the case of activators), or to facilitate the assay or further purification of cells (in the case of antibodies).

[0017] Carriers may be used to alter the behavior of cells during DLD procedures. For example, cells exiting devices at one position may be bound to a carrier to form a complex that exits the same device at a different position. Thus, size based separations may be achieved that would otherwise not be possible. In this case, binding of carrier should be done "in a way that promotes DLD separation." This term, as used in the present context, means that the method must ultimately result in binding that exhibits specificity for a particular target cell type, that provides for an increase in size of the complex relative to the unbound cell of at least 2 μm (and alternatively at least 20, 50, 100, 200, 500 or 1000% when expressed as a percentage) and, in cases where therapeutic or other uses require free target cells, that allows the target cell to be released from complexes by chemical or enzymatic cleavage, chemical dissolution, digestion, due to competition with other binders, by physical shearing, e.g., using a pipette to create shear stress, or by other means. Carriers may also be bound in a way that complements DLD separation.

[0018] In order to determine concentration, cell or particle counts may be made of the target cell or target particle products. Based on this, a determination can be made as to whether to continue recycling product. Depending on the objectives of the party carrying out the process, relative to the concentration in the sample, cells or particles may be concentrated by a factor of at least 3 or by a factor of at least 10. In the case of cells, recirculation in combination with microfluidic processing will preferably be the sole method used for concentration during this process. This is especially true of cells that will be genetically engineered and/or used therapeutically.

[0019] The methods described herein can be used in the separation and concentration of cells derived from bioreactors, flasks, culture plates, culture bags, biological fluids or extracts, and preparations derived from tissues. Particularly preferred are leukocytes (preferably T cells, and most preferably CAR-T cells) or stem cells of a predetermined size. Samples containing these cells may be blood, compositions obtained by performing apheresis, leukapheresis, or leukoreduction, or leukopak preparations and will typically include platelets or red blood cells which are smaller than the predetermined size. Also, cells may be genetically engineered by any method used in the art, including transfection electrically, chemically or by means of nanoparticles or transduction using a virus.

[0020] In some instances, leukocytes or stem cells being recirculated may be bound to a carrier, antibody, or activator in a way that promotes or complements DLD separation. Binding should take place after cells have passed through the microfluidic device at least once and prior to, during or after being recirculated to the microfluidic device. In cases where cells are to be used therapeutically, the process of preparing the cells should not include a centrifugation step and recirculation should be performed until a concentration of cells is obtained that is sufficient to allow them to be therapeutically administered to a patient or for subsequent for processing. Similarly, addition of viral or genetic material in the concentration loop prior to release may be possible. In addition, it is preferred that in cases where a sample is obtained from a patient for the isolation of cells to be used therapeutically, no more than four hours elapse from the time that the obtaining of the sample is complete until processing of cells by DLD is complete.

[0021] It will be readily apparent to those of skill in the art that, with some modifications, the procedures described herein could be adapted to separating target cells or particles from contaminants of a larger size. Apart from being directed to the methods described above, the invention includes the cells (including leukocytes, stem cells and CAR-T cells) or particles produced by the methods.

[0022] Integrated Method for Making Purified Genetically Engineered Target Cells

[0023] In a preferred embodiment, the procedures described above can be used as part of a method for making genetically engineered target cells. In particular, the inven-